

Fig. 1. pH-Dependence of the hydrolysis of CBZ-ALa-Ile catalyzed by the carboxypeptidase (25°C, 50 mM acetate buffer and 50 mM citric acid/ $\text{KH}_2\text{PO}_4$  buffer at pH 2.9 and 3.3)

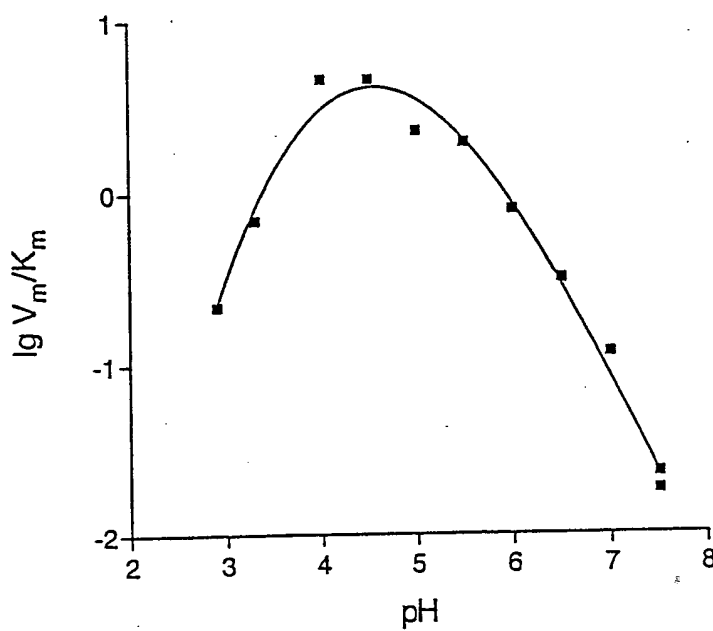
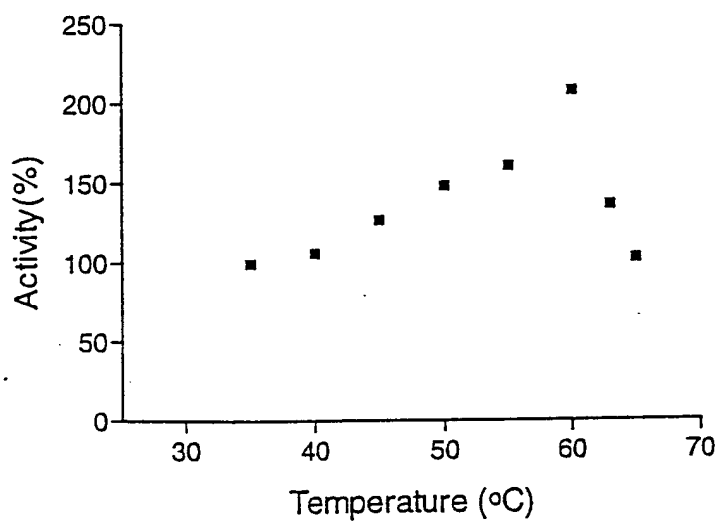


Fig. 2. Temperature optimum of hydrolysis of CBZ-Ala-Ile (1.5 mM) by the carboxypeptidase (50 mM acetic buffer, pH 4.0)



ATCGGTCCCTACGAATTTCTCTCAGTGCTACCCCTTGGTTGACCCAGTTGGCCCTTCCAGGAAGTACACCCCGCTCCGTCCGT  
 MRGYEFLSVLPLVAASWALTPASVGRRL  
 ACCCAAGAACCCACCCGGGTCAAGACTCTTACAACCCGTCACCAATGTCAACCATCCGTACAAGGAACCCCGCCAGAGCCGT  
 PKNPTGVKTLTTANNVTIRYKEPGAEGVCE  
 CCCCCGTGTCAATCCTACTCTGATATGTGACACCTCTCCCGAGTCCCATACCTTCTTCTGTTCTTGAAGCCAGACATA  
 TPGVKSYSGYVDTSPESTPTFWFFEARNPET  
 CCACCTATCACATTGTGGTGAATCGTCCGCTCGAAGCGATTCTTTGATCGGTCTCTTGAAGAGTTGGCCCTTCCATGTC  
 APITLWLNGGPGSDSLIGLFEELGPCHVNSTF  
 TGATGACTACATCAACCTCACTCGTGAAGCGAGTCTCAATTTACTATTCTGTCCAGCCATTGGAGTCCGCTTTTCATA  
 DDYINPHSWNEVSNLLFLSQPLGVGFSSYSDT  
 TTGATCGGTCCATTAACTGTAACTCGGTCGTAATTCGAGCTTTCAGGAGTTCAAGCCCGTACCCAACCATGATG  
 VDGSINPVTGVVENSSFAGVQGRYPTIDATLI  
 GATACTACCAATCTTCCCGCAGAGCCCGCTTCGAGATCTTCAACGATTCTTCTAGTGGACTACCTACCTTGGACTCTACCGTG  
 DTTNLAAEAAWEILOGFLSGLPSLDSRVQSKD  
 CTTCAGTCTATGACCGAGAGCTATCGACCCACTATGTCCTGCACTTCAATCATTTTACGAGCAGAATGAGAGAATCC  
 FSLWTESYGGHYGPAFFNHFFYEONERIANGS  
 TTAATCGTGTTCAGTTAATTTCACTCTCTCGGAATTATTAAACCCATCATCGACGAGCCGATCCAGCCCGCTTACTACCCTG  
 VNGVQLNPNLSLGIINGIIDEAIQAPYYPEFAV  
 AACAACTACCTACCGTATCAAGCTGTCAACGAGACCGTCTCAACTACATGAAGTTGCCAACCAATGCCAAATGGTCCAG  
 NNTYGIKAVNETVYNYMKFANQMPNGCQDLIS  
 CACCTGCAACAGACAAACCCACCCGATTACCTGACTACCCCTCTGCCCGAAGCCACCAACATGTCCAGGACAATGTTGA  
 TCKQTNRTALADYALCAEATNMCRDNVEGPY  
 ACCCTTTCTCGTGGTGGTGTGTATGATATTCGGCATCCATATGATGACCCGACTCCGCCAAGTTATTACAACAAATTTCTGG  
 YAFAGRGVYDIRHPYDDPTPPSYYNKFLAKDS  
 GTTCATGACGCTATCGCGTCAACATCAACTACACCCAGTCCAATAATGACGTCTACTACGCTTTCAGCAACAGCGGACTTT  
 YMDAIGVNINYTQSNNDVYYAFQOTGDFVWPN  
 GTTCATCGAAGACCTCGAGGAGATCCTTGTCTCCCGTGGTGTCTCCCTCATCTATGCCGACCCGATTACATCTGCAACTG  
 FIEDLEEILALPVRVSLIYGDADYICNWFEGG  
 AGCCCGTTTCCCTCGCTCGGAAGTACTCCCAAGCCCGCCAGTTCGAAGCCGAGGTACACCCCGCTGAAAGTCAACCGCGTGG  
 QAVSLAANYSQAAQFRSAGYTPLKVNGVEYGE  
 ACTCCGAGTATGGTAATTTCTCTTCACTCCGCTCTATGAGCCAGCCATGAAGTCCCATACTACCAGCCCATCCCTCCCTG  
 TREYGNFSFTRVYBAGHEVPYYQPIASLQLFN  
 GCGGACTATCTTCGGTTGGATATCCAGAGGCCAGAGAAGATCTGCCAGCTACAAGACGAATGGAACGGCTACAGCTAC  
 RTIFGWIDIAEGQKKIWPSTKNGTATATHTQ  
 CGTCCGTCCCGCTCCCTACCGCTACCGCATGTCCAGTGTGGTATG 1668  
 SSVPLPTATSMSSVGMA

Figure 3: DNA sequence and deduced amino acid sequence of *Aspergillus oryzae* strain ATCC 20386 carboxypeptidase I. Amino acids determined by protein sequencing of purified *A. oryzae* ATCC 20386 carboxypeptidase I are underlined. Primer positions used in PCR are indicated by dotted arrows. Predicted signal sequence peptide is double underlined.

Consensus

	10	20	30	40	50
1	M R G Y E F L S V L P L V A A S W A L P G S T P A S V G R R Q L P K N P T G V K T L T T A N N V T I				
1	- - - - - F V K - - - - -				
1	M R I T S A I A S L L L V - - - - - G T A T S L Q N P H R R A V P A P L T H R S V A S R A V				
1	M L F R S L L S T A V L A V - - - - - S L C T D N A S A A K H G R F G Q K A R D A M N I A N G S A N A V				

Consensus

	60	70	80
51	R - - - - - Y K - - - - - E P G A E G V C E T T - - - - - S Y V D T S P E S H		
1	- - - - - S T K N Y R F L N E K T K A N L V H - - - - - H L P D V I G E M S S L M P I D M H N E		
4	- - - - - P V E R R S N D F E Y L T N K T A R F L V N G T S I - - - - - Q S G Y L S V G S N M N		
42	- - - - - K H S L K I P V E D Y Q F L N N K T K P Y R V E - - - - - S L P D V H F D L G E M S S G L V P I E K G N V		

Consensus

	90	100	110	120
82	- - - - - T F W F E A R H N P E T A P I T L W L N G G P R C S D S L I G L F E E L G P C H - V N S T F D			
43	S R A L F Y I P Q P T I G E P V D E V T I W M N G G P R C C S M E S F L Q E T G R F L W Q P G T Y A			
29	- - - - - M W P W F P E A R N N P Q Q A P L A A W F N G G P R C C S M I G L F Q E N G P C H F V N G D S T			
90	S S L F P W F P S Q N P D A S D E I T I W L N G G P R C C S L D G L L Q E N G P F L W Q P G T Y K			
96	S R S L F F V P Q P T I G E P V D E T T I W L N G G P R C C S L E A L S P G E C R F V W Q P G T Y Q			

Consensus

	130	140	150	160	170
129	D Y I P H S W N E V S L L F L S Q P F L C V C F Y S D T V D G S I N P V T G V V E N S S F A G V				
93	P V E P Y S W V V L T V L W V D Q P F L C V C F Y S I G T P T A T S Q E E T A - - - - -				
77	P S L N N N N Y A M I Y I D Q P F L C V C F Y S Y G T - - - - - D D V T S T V T A P Y V - - - - -				
140	P V P N N N N Y A M I Y I D Q P F L C V C F Y S P G P S T V N D E E D V A - - - - -				
146	P V E P Y S W V V L T V L W V D Q P F L C V C F Y S L G V P T A T S E E E I A - - - - -				

Consensus

	180	190	200	210	220
179	Q G R Y P T I D A T L I D T T N L A A E A A W E I L Q G L S G L P S L D S R V Q S K D F S L W T -				
132	- - - - - P V E P Y S W V V L T V L W V D Q P F L C V C F Y S V K F F K N F Q K T Y G I K N F K I Y V T				
119	- - - - - P S L N N N N Y A M I Y I D Q P F L C V C F Y S Y A Q R P E Y E S R - - - - - D F A I F T -				
179	- - - - - P V P N N N N Y A M I Y I D Q P F L C V C F Y S N S W F K H F V D T F D L H G R K V Y I T				
185	- - - - - P V E P Y S W V V L T V L W V D Q P F L C V C F Y S V K F F K N W Q Q I F G I K N F K I Y V T				

Consensus

	1	10	20	30	40	50
1	M R G Y E F L S V L P L V A A S W A L P G S T P A S V G R R Q L P K N P T G V K T L T T A N N V T I					
1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
1	- - - - - F V K - - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
1	M R I T S A I A S L L L V - - - - - G T A T S L Q N P H R R A V P A P L T H R S V A S R A V					
1	M L F R S L L S T A V L A V - - - - - S L C T D N A S A A K H G R F G Q K A R D A M N I A N G S A N A V					

## Consensus

[illegible]

## Consensus

F . . . F			W . N G G P G . . . S			110			120		
90			100			110			120		
82	-	-	T F	E A R H N P E T A P I T L	L N G G P G	S D S	L I G L F E E L G P C H - V N S T F D				
43	S	R A L	T F	Q P T I G E P V D E V T I	W M N G G P G	C S S	M E S F L Q E T G R F L W Q P G T Y A				
29	-	-	M W	E A R N N P Q Q A P L A A	W F N G G P G	C S S	M I G L F Q E N G P C H F V N G D S T				
90	S	S L	F F	P S Q N P D A S D E I T I	W L N G G P G	C S S	L D G L L Q E N G P F L W Q P G T Y K				
96	S	R S L	F F	Q P T I G E P V D E T T I	W L N G G P G	C S S	L E A L S P G E C R F V W Q P G T Y Q				

## Consensus

	N . . . N	SW . . . N	N . . . N	Q P . G . G . S	150	160	170	
	130	140	150	160	170			
129	DYI	NEVS	LLFLS	OLFLS	LLFLS	YSDTVDGSI	NPVTGVVEN	SSFAGV
93	PVE	VVLT	VVLWVD	OLVWVD	YSGTPTAT	SGTPTAT	SEETAT	- - -
77	PSL	NNYA	MIYID	OLYID	YGGT- - -	YGGT- - -	DDVTSTVTA	APV - -
140	PVP	TNLT	VVYID	OLYID	PGPSTVN	PGPSTVN	DEEDVA - -	- - -
146	PVE	VNLT	VVLWVD	OLVWVD	LGVPPTAT	LGVPPTAT	SEEEIA - -	- - -

✓

[illegible]

. E S Y . G . Y . P . . . . . G . . . . .																												Consensus										
240																												250	260	270								
228	-	E S Y	G	A	F	F	N	H	F	Y	E	Q	N	E	R	I	A	N	G	S	V	N	V	Q	L	N	F	S	L	G	I	I	D	E	A	I	Q	A.oryzae CP1
156	G	E S Y	A	F	F	N	H	F	Y	E	Q	N	E	R	I	A	N	G	S	V	N	V	Q	L	N	F	S	L	G	I	I	D	E	A	I	Q	Penicillium S3	
142	-	E S Y	G	A	F	F	N	H	F	Y	E	Q	N	E	R	I	A	N	G	S	V	N	V	Q	L	N	F	S	L	G	I	I	D	E	A	I	Q	Penicillium S1
203	G	E S Y	A	F	F	N	H	F	Y	E	Q	N	E	R	I	A	N	G	S	V	N	V	Q	L	N	F	S	L	G	I	I	D	E	A	I	Q	A.phoenicis	
209	G	E S Y	A	F	F	N	H	F	Y	E	Q	N	E	R	I	A	N	G	S	V	N	V	Q	L	N	F	S	L	G	I	I	D	E	A	I	Q	A.niger	

F . . . . . N . . . . .										Consensus																																									
280										290										300										310																					
277	A	P	Y	P	E	-	-	-	-	A	V	N	T	Y	-	-	G	I	K	A	V	N	E	T	V	Y	N	Y	M	K	F	A	N	Q	M	P	N	G	C	Q	D	L	I	S	T	C	A.oryzae CP1				
204	V	P	F	V	K	E	N	A	N	L	F	N	F	N	E	T	F	M	A	E	L	E	H	L	H	K	S	C	-	G	Y	A	D	F	I	D	K	Y	L	T	F	P	P	K	E	Q	P	P	Penicillium S3		
191	E	K	A	Y	I	D	-	-	-	-	F	S	Y	N	S	Y	-	-	Q	-	Q	I	I	D	S	S	T	R	D	-	-	S	L	D	A	Y	N	N	Q	C	L	P	A	L	Q	Q	C	Penicillium S1			
250	A	V	R	H	L	N	H	Y	N	N	I	F	R	L	N	S	T	F	L	S	Y	I	N	G	K	A	D	K	C	-	G	Y	N	A	F	L	D	K	A	I	T	Y	P	P	P	T	P	F	P	T	A.phoenicis
257	V	P	F	V	Q	K	N	N	A	L	F	N	F	N	A	S	F	L	A	E	L	E	S	I	H	E	Q	C	-	G	Y	K	D	F	I	D	Q	Y	L	V	F	P	A	S	G	V	Q	P	P	A.niger	

C . . . . . V . . . . . P . . . . .										Consensus				
320	330	340	350	360										
320	K Q T N R T A L A D - - - Y A L C A E A T - - - N M	C R D N V E G P Y Y A F A G R G	V Y D I R H	P Y	A.oryzae CP1									
253	L F F N Y T S M A N E D V F D M V Y N E V F K I N P	C F D L Y E E V N L M C P L Q W D	L A F - -	T	Penicillium S3									
231	S Q S G S T S D - - - - - C T N A D - - - S V	C Y Q N I E G P I S S S G D F D	Y D I R E	P S	Penicillium S1									
299	A P - - - E I T E D C Q V W D E V V M A A Y D I N P	C F N Y Y H L I D F C P Y L W D	L G F - -	S	A.phoenicis									
306	K A M N W S D - P T C D V Y D I V N N A V L D P N P	C F N P Y E I N E M C P I L W D	L G F - -	T	A.niger									

		Consensus																								
		370					380					390					400					410				
364	DDPT	PSY	YNK	FFL	AKD	SV	MD	AI	-	GV	NI	NY	TQ	SN	ND	VY	AF	QQ	TG	DF	VW	PN	A.oryzae CP1			
301	SLVY	QPA	GAT	VYF	DRAD	VKK	KAL	HA	PN	VTV	WA	EC	SN	NP	VF	VGG	SS	GP	EQ	EG	D	Penicillium S3				
270	NDPY	PKTY	STYL	SDPT	VVK	AI	-	GART	NY	QEE	C	PN	GP	YN	KFA	ST	G	DN	PRS	-	-	Penicillium S1				
344	-	LGF	-	-	GPD	NY	FN	RS	DV	QKI	LH	VPP	TD	YS	VC	SE	TV	IF	ANG	DGS	-	-	DPS	A.phoenicis		
353	EVDY	LPA	APAS	TLT	-	ALIK	RAM	HA	PN	IT	WS	EC	SV	ES	VF	VGG	DGG	PE	OE	GD	-	-	A.niger			